

Agrobacterium tumefaciens strain GV3101 by electroporation. Transgenic plants were generated by *in planta* transformation (Bechtold *et al.*, 1993), using the NPT II gene as a selectable marker to identify transgenic plants.--

IN THE DRAWINGS

Please insert attached Figures 9 and 10 to the end of the specification.

IN THE SEQUENCE LISTING

Please replace the Sequence Listing as originally filed with the attached substitute Sequence Listing in its place.

REMARKS

The specification is corrected and a substitute Sequence Listing and Figures 9 and 10 are herein submitted to correct obvious errors and to coordinate the SEQ ID NOS with the order they are set forth in the specification.

As background, this application is based from PCT Application No. PCT/AU99/01079 which claims priority to Australian Provisional Application No. PP7469 dated December 3, 1998 and to U.S. Provisional Application No. 60/116,928 dated January 21, 1999. In particular and in regards to the drawings, Applicants submit that Figures 9 and 10, although omitted from the instant application, are indeed subject matter of this application based on their inclusion in second priority filing and reference to them in the instant originally filed specification. Applicants submit that the request for filing of the U.S. Provisional 60/116,928 confirms that 12 sheets of drawings were filed, i.e., that Figures 9 and 10 were indeed filed with the U.S.

Provisional Application. Additionally, the Brief Description of the Drawings on pages 16 and 17 of this application do include brief descriptions of Figures 9 and 10, and such are additionally referred to in the first full paragraph on page 43 of the specification. Therefore, omission of Figures 9 and 10 from this application was an oversight, Figures 9 and 10 were disclosed in the U.S. priority filing and were referenced in this specification. Accordingly, no new matter is being added and consideration and acceptance of these Figures is respectfully requested.

As further background, the existing Sequence Listing filed with the original specification, consists of 31 pages. It can be seen that page 1 to 10 of the original filed Sequence Listing were prepared using PatentIn Release #1.0, Version #1.30 (EPO). Pages 1 through 10 set out SEQ ID NOS.: 1-3. However, at the bottom of page 10 it can be seen that additional sequence numbers 4 through 25 were intended to be inserted. Pages 11 to 31 of the original filed Sequence Listing were prepared using PatentIn Version 2.1. Sequences 1-10 on pages 11 through 15 are DNA sequences from *Brassica napus*. Sequences 11-25 on pages 16 through 30 are the corresponding amino acid sequences from *Brassica napus*. Consequently, Applicants submit that the errors in the sequence listing largely result from the change from an earlier version of PatentIn to one which was used at the time of filing of the specification.

In particular, SEQ ID NO:26 on page 30, SEQ ID NOS.: 4, 5 and 26 through 30 were listed out of order in the original sequence listing filed with the original specification. Additionally, SEQ ID NOS.: 26 through 30 in the original sequence listing were incorrectly identified as *Brassica* sequences when in fact they are *Arabidopsis* sequences as clearly set forth in Example 22 on page 50 of the originally filed specification. SEQ ID NOS.: 31 through 42 were disclosed in the specification as filed and are herein identified as such to comply with

requirements for patent applications containing nucleotide and amino acid sequence disclosures.

No new matter is added in the application.

Attached for your convenience is a table summarizing the Sequence Listing provided herewith, the errors it corrects, and the location of the sequences within the originally filed specification.

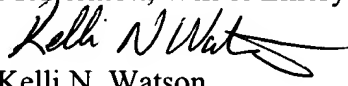
Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The changes to the specification are underlined. The attached page is captioned "Version With Markings To Show Changes Made."

In addition, hereto is an attached the substitute Sequence Listing in paper and computer readable format. The paper copy and computer readable copy of the substitute Sequence Listing are the same. The substitute Sequence Listing does not include new matter.

CONCLUSION

Entry of the substitute Sequence Listing and Preliminary Amendment and favorable consideration are respectfully requested.

To the extent necessary, please grant any extension of time deemed necessary for entry of this communication. Please charge any deficient fees, or credit any overpayment of fees, to Deposit Account 500417.

Respectfully submitted,
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DATE: December 12, 2001

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ATTACHMENT¹

Version With Markings To Show Changes Made

IN THE SPECIFICATION

The last paragraph of page 16 continuing onto page 17 is replaced with the following rewritten paragraph in its place.

--Figure 9 (SEQ ID NO: 4) shows the partial genomic sequence of an *FLF-like* sequence from *Brassica napus*, showing the location of exons, and the predicted sequence of the translated product.--

The first full paragraph on page 17 is replaced with the following rewritten paragraph in its place.

--Figure 10 (SEQ ID NO: 5) shows a comparison of the predicted translated product of a *Brassica napus FLF-like* sequence (top lines), and the predicted *FLF* translation product from *Arabidopsis thaliana*, showing identical amino acids (|), highly conserved amino acids (:), and conserved amino acids (.)--

The first paragraph of page 44 is replaced with the following rewritten paragraph in its place.

--The cDNA sequences and predicted amino acid sequences are set out in SEQ ID NOS: 6 to 15 and SEQ ID NOS: 16 to 25[3] respectively. The sequences probably represent 5 genes, grouped as follows:

12.1/16.1

15.1/16.2/18.2

11.2

14.1/18.1/20.1

11.3

The partial genomic sequence and translation in Figure 9 correspond to the cDNAs 12.1/16.1.--

The first full paragraph of Example 5 on page 22 is replaced with the following rewritten paragraph in its place.

--Example 5 Construction of 35S::gene A plasmid

As the larger of the initially isolated gene A cDNA clones lacked the AT of the ATG of the start codon, oligonucleotide-directed mutagenesis was employed to generate a 200bp fragment from the 5' end of the cDNA which contained the absent nucleotides. Two oligonucleotides were synthesized on an Applied Biosystems DNA Synthesizer for this purpose: [-]

(1) (SEQ ID NO: 31)

5' AAGCCGCGGACAATGGAAGCTGTAAGATGC 3'

(2) (SEQ ID NO: 32)

5' GAGAGGCTGGTTAACCGGAG 3'.--

Example 6 of page 23 is replaced with the following rewritten example in its place.

--Example 6 Construction of 35S::gene B plasmid

A binary construct containing gene B under the control of the CaMV 35S promoter was generated by cloning a XhoI/SpeI digested PCR product, amplified using the gene

B cDNA clone as template with the primers, using methods similar to those described in

Example 5:

(SEQ ID NO: 33) 5' CCGCT**CGAG**CTTAGTATCTCCGGCG 3' and

(SEQ ID NO: 34) 5' GGACTAG**TCGCC**CTTATCAGCGGA 3',

in which restriction sites are shown in bold, and the sequence hybridizing to gene B cDNA is underlined, into XhoI/SpeI digested pART7 (Gleaves, 1992) containing the CaMV 35S promoter. The 35S::gene B cassette was then subcloned using NotI into pART27 (Gleaves, 1992) and introduced into *A. tumefaciens* strain GV3101 (Koncz and Schell, 1986) as described above. Transgenic plants were generated by *in planta* transformation (Bechthold et al, 1993).--

The first full paragraph on page 24 (part of Example 7) is replaced with the following rewritten paragraph in its place.

--The NPTII probe was generated as described above. The 3' Ac probe was a SphI fragment (Lawrence *et al*, 1993), and probe 4 was generated by amplification of the wild-type genomic clone with primers:

(SEQ ID NO: 35) 5' -GTATAGGGCACATGCCC-3' and

(SEQ ID NO: 36) 5' -CACTCGGAGCTGTGCC-3'.--

The first paragraph of Example 13 on page 30 is replaced with the following rewritten paragraph in its place.

--Example 13 Anti-Sense Constructs

Anti-sense plant constructs have been generated using an anti-sense *FLF* gene construct under the control of the CaMV 35S promoter. A 35S::*FLF* antisense binary construct was generated by cloning the EcoRI/SpeI digested PCR product amplified with primers

(SEQ ID NO: 37) CGGAATTCTCACACGAATAAGGTAC and

(SEQ ID NO: 38) GGACTAGTGGTCAAGATCCTTGATC

as described for the 35S::*FLF* construct. This amplified the region downstream of the MADS box, so that the antisense construct lacks the MADS box region. The PCR product was cloned into pART7 and pBART 27 (which is a derivative of pART27), and transgenic plants were generated as described above, except that the *Bar* gene was used as the selectable marker.--

The first full paragraph on page 43 is replaced with the following rewritten paragraph in its place.

--The partial genomic sequence of the *Brassica napus FLF-like* gene is set out in SEQ ID NO. 4 (Figure 9), and the amino acid sequence of the predicted translation product is set out in SEQ ID NO. 5 (Figure 10). The partial genomic sequence, showing the location of exons and the sequence of the corresponding translated product, is illustrated in Figure 9, and the sequence of the predicted translated product from the *Brassica napus* gene is compared with the corresponding product from *Arabidopsis thaliana FLF* in Figure 10. There is a high degree of conservation, with 79% identity and 83% similarity in the deduced FLF protein sequence (as determined by the University of Wisconsin Genetics Computer Group software package version 9.1, using default parameters).--

The second paragraph on page 51 (continuing onto page 52) is replaced with the

following paragraph in its place.

--cDNA from a chromosome 1 *FLF* -like gene (*FLF-LIKE1*) was isolated using a RT PCR based method. First strand cDNA was generated from 5 µg of Col-0 total RNA. Reactions were carried out using Superscript II (GIBCO BRL) in a 20 µl volume according to the manufacturer's instructions. *FLF-LIKE1* transcript was amplified by PCR using 1µl of the first strand cDNA synthesis reaction as template with primers :

(SEQ ID NO: 39) 5'-ATTGAATTCGGGCATAACCCTTATCGGAGATTTG-3' and

(SEQ ID NO: 40) 5'-AACGGATCCGTTGATGATGGTGGCTAATTGAGCAG-3';

Eco RI and Bam HI restriction sites respectively are underlined. The amplification reaction was carried out in a final volume of 40 µl, which contained 2.5 µM of each oligonucleotide primer, 1.0 units of Amplitaq Polymerase (Perkin Elmer) and 250 µM of each of the four deoxynucleotides. Conditions for amplification were as follows: 94°C for 2 min, 40 cycles consisting of 15 s denaturation at 94°C, annealing at 55°C for 15 s and polymerisation at 72°C for 1 min, and a final extension at 72°C for 4 min before the temperature was decreased to 25°C. PCR products were purified using QIAquick PCR purification kit (Qiagen), digested with restriction enzymes Eco RI/Bam HI, and ligated into the corresponding restriction sites of a pBIISK+ vector (Stratagene). Positive colonies were sequenced using universal primers with the Applied Biosystems Big Dye terminator sequencing mix according to the manufacturer's instructions, and analysed using an Applied Biosystems 377 sequencing machine (Perkin Elmer). cDNA sequences obtained were compared to *Arabidopsis* genomic sequence (BAC F22K20; AC002291). The University of Wisconsin GCG software package was employed for sequence analysis.--

The second paragraph on page 52 is replaced with the following paragraph in its place.

--A binary construct containing the *FLF-LIKE1* cDNA under the control of a *CaMV 35S* promoter was generated by cloning an Eco RI/Kpn I digested PCR product into an Eco RI/Kpn I pART7 vector (Gleave, 1992) containing a *CaMV 35S* promoter. The PCR product was amplified using 200 pg of the *FLF-LIKE1* cDNA clone as template with primers:

(SEQ ID NO: 41) 5'-ATTGAATTCGGGCATAACCCTTATCGGAGATTTG-3' and

(SEQ ID NO: 42) 5'-CTAGTGGTACCGTTGATGATGGTGGCTAATTGAGC-3';

Eco RI and Kpn I restriction sites respectively are underlined. The amplification reaction was carried out as described above. The cloned PCR product was sequenced to ensure that no mutations had been introduced during the amplification procedure. The *35S::FLF-LIKE1* cassette was then subcloned into pART27 using Not I (Gleave, 1992), and introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation. Transgenic plants were generated by *in planta* transformation (Bechtold *et al.*, 1993), using the NPT II gene as a selectable marker to identify transgenic plants.--

IN THE DRAWINGS

Figures 9 and 10 are attached hereto.

Applications corresponding to PCT/AJ99/01079

| New SEQ ID NO. (provided with this e-mail) | SEQ ID NO as filed with PCT | Page of PCT where sequence is described or listed | Comments |
|-----------------------------------------------|-----------------------------|---------------------------------------------------|------------------------------------------------------------------------------------------------------|
| 1 | 1 (first series) | 26 | Arabidopsis DNA |
| 2 | 2 (first series) | 26 | Arabidopsis cDNA |
| 3 | 3 (first series) | 26 | Arabidopsis protein |
| 4 | 26 (second series) | 43 | Brassica partial genomic sequence. Sequence also set out in Figure 9. |
| 5 | 27 (second series) | 43 | Brassica Sequence also set out in Figure 10 |
| 6 | 1 (second series) | 44 | |
| 7 | 2 (second series) | 44 | |
| 8 | 3 (second series) | 44 | |
| 9 | 4 (second series) | 44 | |
| 10 | 5 (second series) | 44 | |
| 11 | 6 (second series) | 44 | |
| 12 | 7 (second series) | 44 | |
| 13 | 8 (second series) | 44 | |
| 14 | 9 (second series) | 44 | |
| 15 | 10 (second series) | 44 | |
| 16 | 11 (second series) | 44 | |
| 17 | 12 (second series) | 44 | |
| 18 | 13 (second series) | 44 | |
| 19 | 14 (second series) | 44 | |
| 20 | 15 (second series) | 44 | |
| 21 | 16 (second series) | 44 | |
| 22 | 17 (second series) | 44 | |
| 23 | 18 (second series) | 44 | |
| 24 | 19 (second series) | | typographical error in specification, at page 44 line 3 should read "NOS: 16 to 25 respectively."... |

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| | | | |
|----|--------------------|-------------|-------------------------------------------------------------------------------------------------------------------------------|
| 25 | 20 (second series) | | error in specification, at page 44 line 3 should have read "NOS: 16 to 25 respectively."... |
| 26 | 21 (second series) | 50 | specification correctly lists sequence as SEQ ID 26 Incorrectly identified as <i>Brassica</i> sequence in original listing |
| 27 | 25 (second series) | 50 | specification correctly lists sequence as SEQ ID 27 Incorrectly identified as <i>Brassica</i> sequence in original listing |
| 28 | 22 (second series) | 50 | specification correctly lists sequence as SEQ ID 28 Incorrectly identified as <i>Brassica</i> sequence in original listing |
| 29 | 23 (second series) | 50 | specification correctly lists sequence as SEQ ID 29 Incorrectly identified as <i>Brassica</i> sequence in original listing |
| 30 | 24 (second series) | 51 | specification correctly lists sequence as SEQ ID 30 Incorrectly identified as <i>Brassica</i> sequence in original listing |
| 31 | not listed | p22 line 13 | |
| 32 | not listed | p22 line 14 | |
| 33 | not listed | p23 line 18 | |
| 34 | not listed | p23 line 19 | |
| 35 | not listed | p24 line 8 | |
| 36 | not listed | p24 line 9 | |
| 37 | not listed | p30 line 27 | |
| 38 | not listed | p30 line 28 | |
| 39 | not listed | p51 line 32 | |
| 40 | not listed | p51 line 33 | |
| 41 | not listed | p52 line 24 | |
| 42 | not listed | p52 line 25 | |